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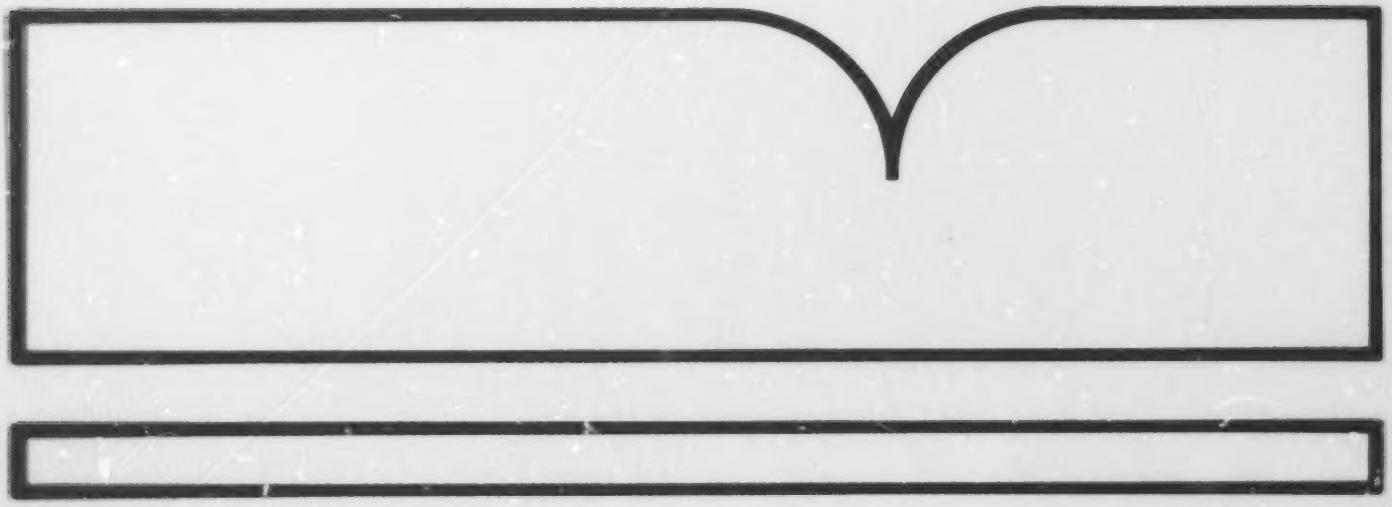

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ABSTRACT

We conducted a series of laboratory pulse-exposure experiments to model short-term field exposures of two representative estuarine crustaceans, Penaeus duorarum and Mysidopsis bahia, to the organophosphate insecticide fenthion. These tests established acutely lethal and and nonlethal concentrations during pulse exposures. The data were necessary for interpretation of responses of test animals in the field when fenthion concentrations changed rapidly with time.

The toxicity of fenthion to caged pink shrimp, mysids, and sheepshead minnows (Cyprindon variegatus) was determined in the field following two aerial applications, separated by 72 h, to control adult saltmarsh mosquitoes. At one estuarine site, initial concentrations of fenthion in water were 1.5 µg/ℓ following Spray 1 and 0.29 µg/ℓ after Spray 2. Within 12 to 24 h, however, fenthion was not detectable (<0.01 µg/ℓ) because of rapid tidal flushing and high dilution at this site. Although initial exposures approached or exceeded laboratory 24-h LC50's for pink shrimp (0.40 µg/ℓ) and mysids (0.42 µg/ℓ), no mortality occurred among caged animals. At a second site along a residential saltwater canal with limited tidal flushing and dilution, initial concentrations of fenthion were 2.6 µg/ℓ (Spray 1) and 0.5 µg/ℓ (Spray 2). Within 12 to 24 h post-spray, fenthion decreased to 0.4 µg/ℓ (Spray 1) and 0.14 µg/ℓ (Spray 2) and continued to diminish during the next 48 to 72 h. These concentrations approximated the 48- and 72-h LC50's for pink shrimp (0.22 µg/ℓ and 0.15 µg/ℓ) and mysids (0.37 µg/ℓ and 0.18 µg/ℓ). All exposure concentrations were three orders of magnitude below the 24-h LC50 (1,900 µg/ℓ) for sheepshead minnows and no mortality occurred among caged fish. By deploying caged pink shrimp and mysids daily, before and after each spray, we varied in situ exposure

regimes and obtained responses among caged test populations that ranged from no observed effect to 100% mortality.

The responses of caged pink shrimp and mysids exposed to slowly changing concentrations of fenthion in the field were similar to what would have been predicted based on laboratory tests that established 24-, 48-, and 72-h LC50's. Laboratory pulse-exposure tests were predictive of no-effect and effect pulse exposures in the field. These comparisons demonstrated that predictions of fenthion toxicity based on laboratory test results were valid when field and laboratory exposure regimes were similar.

Key Words: Field validation, acute toxicity, fenthion, pulse-exposures, shrimp, mysid, sheepshead minnow, aquatic toxicology

INTRODUCTION

Toxicolgical data that accurately describe exposure-response relationships for nontarget species are a necessary component of field-validated risk assessments for pesticides. Acute toxicity test results obtained from standard laboratory tests, such as the ASTM Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (E 729-80), are among the most common and cost-effective data used in pesticide risk assessment. Laboratory exposure concentrations and test conditions are selected to obtain replicable and definitive acute toxicity data. However, laboratory test results may not apply to nontarget species exposed during pesticide application or from subsequent aerial drift or surface runoff. Although all factors that affect an organism's exposure and sensitivity to a pesticide cannot be accounted for, individually, in risk assessment it is necessary to determine whether laboratory data may be used to predict environmental effects under actual-use conditions.

Fenthion (0,0-dimethyl 0-[3-methyl-4-(methylthio)phenyl] phosphorothioate) is an organophosphate insecticide registered for mosquito and fly control. More than 38,600 ℓ of fenthion were used in Florida between June 1982 and June 1984, the majority for saltmarsh mosquito control[1]. When applied as an aerial thermal fog, fenthion is mixed with diesel fuel (1:20 v/v) to facilitate application and enhance dispersion. The nominal application rate of 32.5 g fenthion per hectare (0.029 lb/acre) would yield an aqueous concentration of 11 µg/ℓ, assuming 100% of the pesticide was deposited uniformly over an aquatic habitat 0.3 m deep.

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[1]Personal Communication: Office of Entomology, FL Dept. Health and Rehabilitative Services, Jacksonville, FL.

This environmental concentration of fenthion, a worst-case prediction, exceeds laboratory 24-h and 96-h LC50's for pink shrimp (Penaeus duorarum) (0.40 µg/ℓ and 0.11 µg/ℓ), and mysids (Mysidopsis bahia) (0.42 µg/ℓ and 0.15 µg/ℓ), but does not exceed the 24- or 48-h LC50's for sheepshead minnows (Cyprinodon variegatus) (1,900 µg/ℓ and 1,900 µg/ℓ) (1). For the application rates and assumptions stated above, the maximum predicted diesel fuel concentration of 220 µg/ℓ would be approximately 5 times less than the 96-h acute LC50 of diesel fuel for mysids (0.88 µℓ/ℓ)[2] or grass shrimp (Palaemonetes intermedius) larvae (1.4 µℓ/ℓ) (2) and would not likely be acutely lethal to caged invertebrates.

Previous field studies have shown that fenthion concentrations in estuarine habitats change rapidly (1). Therefore, laboratory tests that simulate field exposures were needed in order to predict field effects more accurately. We determined the responses of mysids to laboratory pulse exposures of fenthion as an additional basis for predicting field exposure-response relationships. We also studied effects of fenthion on estuarine animals following aerial thermal fog applications of the insecticide to control saltmarsh mosquitoes. Field exposure regimes were characterized and acute toxicity to pink shrimp, mysids, and sheepshead minnows was quantitated for animals caged in an estuarine habitat. Habitats with different flushing and dilution characteristics were selected to evaluate animal responses to pulse exposures as well as responses to prolonged exposures of more slowly changing concentrations that might approximate exposure regimes in routine laboratory toxicity tests. By selecting contrasting habitats and by deploying caged animals before, during, and after pesticide applications, we obtained a variety of responses

[2]Personal Communication: P.R. Parrish, US EPA, Gulf Breeze, FL 32561.

that ranged from no effect to 100% mortality in test populations of pink shrimp and mysids. To determine the applicability of standard laboratory test results and demonstrate whether predictions of fenthion toxicity based on such tests would be valid, exposure regimes and animal survival in the field were compared with laboratory-derived LC50's.

MATERIALS AND METHODS

Laboratory Pulse-Exposure Tests with Mysids

Five toxicity tests incorporating pulse exposures to fenthion were conducted with laboratory-reared, juvenile M. bahia, 24-to 48-h old. Tests were conducted with filtered (20 µm) seawater at 22° C, 20 °/oo salinity, pH 7.6 to 8.0, and 4.8 to 7.2 mg dissolved oxygen/ℓ. Test vessels were 60-ℓ aquaria equipped with self-starting overflow siphons that provided 20 ℓ drawdown. Two 1-ℓ beakers, fitted with two screened (450-µm nylon mesh) openings, 3-cm diameter, 1 cm above the base and on opposite sides of the beaker, were placed in each aquarium and 10 mysids added to each beaker. This configuration confined mysids for ease of counting yet ensured exchange of water within beakers. Three exposure concentrations and a carrier control (triethylene glycol) were tested in each experiment. Pulsed exposures consisted of a 2-h period when stock solutions (or carrier alone) were injected from a syringe pump (20 mℓ/h), mixed with influent seawater supplied at 30 ℓ/hr, and introduced into each aquarium. Injection of fenthion was then stopped and test concentrations were diluted with uncontaminated seawater for 94 h at 30 or 15 ℓ/h. Different dilution rates were chosen to vary total exposure at similar maximum concentrations. Water samples were taken hourly for 4 h, then a single sample was taken 8 h after test initiation to characterize the rapidly changing exposure regimes. Fenthion concentrations were

modeled from theoretical exchange rates (3), which indicated that exposure concentrations would be reduced by 95 to 99% from maximum concentrations in 12 h. Mortality was monitored at 12, 24, 48, 72, and 96 h. Experimental procedures were similar to those employed by Borthwick et al. (1) for testing pink shrimp.

Field Studies

Fenthion application - Effects of fenthion applied as an aerial thermal fog for control of adult saltmarsh mosquitoes were studied in cooperation with the Collier County, FL, Mosquito Control District during routine applications. Fenthion was applied as a 20:1 mixture of diesel fuel and Baytex® Liquid Concentrate (Mobay Chemical Co.) from DC3 aircraft at a nominal rate of 32.5 g active ingredient per hectare (0.029 lb/acre). Residential areas were fogged with fenthion on June 20 and 23, 1984, between 6:30 AM and 7:30 AM to coincide with early morning activity of the mosquitoes. Although mosquito control was intended for land areas, fenthion entered aquatic habitats as a result of drift or direct depositon following application over land along residential canals.

Study sites - Two treated sites within the mosquito control treatment area and one untreated reference site were studied. Both treated sites were fogged as part of the same flight pattern for fenthion application. Site 1 was within the Isles of Capri development at a homeowner's dock along Johnson Bay, approximately 1 km from Capri Pass and the Gulf of Mexico. Field tests at this site were conducted in 1.5 m water over a firm bottom of sand and clay with soft, calcium cabonate floc. Because of its proximity to the Bay and Gulf, there was substantial tidal flushing,

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mixing, and dilution with uncontaminated water at this site.

The second treated site was at a residential dock along a canal within the Marco Island development, approximately 2.5 km by canal from the Marco River. Water sampling and animal deployments were conducted in 1.5 m to 2 m water over a soft silt and muck bottom. The canal, with concrete seawalls on both sides, was 13 m wide with a maximum water depth 4 to 5 m. Since this site was distant from the mouth of the canal system and the canal system permeated the island development, tidal exchange and dilution with uncontaminated water following a spray was less than that at Site 1. A reference site, not treated with fenthion, was located within the Rookery Bay National Estuarine Sanctuary. Sampling at this site was conducted from a dock at the Shell Island Field Laboratory at Rookery Bay. The site had a silt and sand bottom, 1.3 m depth, and open tidal exchange with the waters of Rookery Bay.

Experimental design - Pink shrimp and mysids were deployed daily at all three sites, beginning the day before fenthion treatment and continuing until post-treatment deployments demonstrated no significant ($\leq$ 10%) mortality in 48 h. Pre-treatment survival, survival of exposed animals, and post-treatment survival were compared to determine the acute effect of fenthion on caged animals at individual sites. Pre-treatment groups were exposed to fenthion along with newly deployed animals. Because expected environmental concentrations of fenthion were orders of magnitude less than laboratory LC50's for sheepshead minnows, fish were deployed only on the day prior to a spray and left for observation for 5 days. Treated-site survival was compared with that at the reference site to ensure that test animals deployed were healthy. Water samples for fenthion analyses were collected at 12-h intervals, except following a thermal fog treatment

when 0.5-, 1-, 2-, 4-, 6-, 9-, 12-, 18-, and 24-h post-treatment samples were collected. For each batch of animals deployed, daily fenthion exposure regimes were matched with daily survival data and compared to laboratory acute toxicity test results. Specific procedural steps are summarized below.

Analyses of water samples - Water samples for fenthion analyses were collected in 1-ℓ glass bottles fitted with water inflow and air displacement vents to minimize water column turbulence during filling. At each sampling, water was collected from 4 cm below the surface and 10 cm above the bottom, at sites of animal cages. Water samples were stored on ice and transported to the field laboratory within 3 h. Each water sample was drawn through a resin column (a 6-mm ID pasteur pipette filled with 2.3 g XAD-4® resin) at 15 mℓ/min by a peristaltic pump. Sample resin columns were held at 4°C and shipped on ice to the Environmental Research Laboratory at Gulf Breeze (ERL/GB) for elution and quantitation. Daily field-spike samples (1 µg fenthion/ℓ control water) and triplicate samples taken at sprayed sites at 2, 4, and 12 h post-spray assured precision and acccuracy of our measures of environmental concentrations of fenthion.

Upon receipt at ERL/GB, resin columns were eluted with 15 mℓ of Nanograde® acetone (Burdick Jackson Laboratories, Muskegon, MI). The eluate was concentrated to approximately 1 mℓ on an N-Evap® with a gentle stream of nitrogen. Isooctane was added (3 mℓ), water removed, and sample volume adjusted to 5 mℓ. Sample extracts were injected into a Hewlett-Packard 5710 gas chromatograph with dual N-P detectors according to procedures described by Lores et al. (4). These procedures provided a detection limit of 0.01 µg fenthion/ℓ and average recovery efficiencies of 82% (± 10%) for field-spiked samples and 98% (± 5%) for laboratory-spiked

samples.

Test animals - Pink shrimp used during the field study were purchased from a bait shrimp distributor at Bonita Springs, FL, and transported to the field laboratory at the reference site in 64-ℓ ice chests containing approximately 60 animals per chest in seawater that was continuously aerated. Upon arrival, water volume in each ice chest was reduced to approximately 10 ℓ and then refilled with reference site water at 5 ℓ/hr. Water was continuously aerated during holding, except during the night of 23 June when the aeration system failed and approximately 40% of the pink shrimp died. For all other batches of pink shrimp, mortality was less than 15% during the 12- to 36-h holding period before deployment.

Each morning, 80 shrimp were transported to each study site and placed, 10 per cage, in 4 surface and 4 bottom cages. Pink shrimp cages were constructed of clear plastic, formed into a 51 x 12 x 13-mm rectangle with 10 partitioned compartments and open sides that were screened with 1.8-mm mesh Nitex® (5). The styrofoam float on surface cages was removed from the lid when cages were used for bottom exposures. Shrimp were observed daily at 12-h intervals and 6 h after each spray. Pink shrimp lengths averaged 63 mm and ranged from 44 to 89 mm, rostrum to telson.

Mysids and sheepshead minnows were reared at ERL/GB and transported by automobile or air to the field laboratory in oxygenated seawater in plastic bags within ice chests. At the field laboratory, these animals and the water used in transport were transferred to polypropylene buckets and the water used in transport was replaced with local water by partial replacements over 24 h. Mysids and sheepshead minnows were fed live brine shrimp nauplii and the holding water was partially replaced at least daily. Mysids were held from 2 to 4 days at the field laboratory before

deployment; sheepshead minnows, either 2 or 5 days.

Mysid and sheepshead minnow cages were constructed by cutting the bottom 2 cm and the top 3.5 cm off widemouthed polypropylene jars (8 cm ID) and attaching them to the end of a 14-cm high X 8-cm OD cylinder of nylon mesh with silicone adhesive. The mesh size was 450 µm for mysid cages and 600 µm for sheepshead minnow cages. A nylon cord attached to the rim of the cage lid was used to tether the cages to submerged bricks so that cages floated with their top side flush with the surface of the water.

On the evening before deployment of cages, populations of mysids and sheepshead minnows were randomly divided into 3 groups of 30 to 40 animals. These groups were placed in incubation cups that were constructed by attaching a 24-cm high cylinder of 363-µm nylon mesh to the inside walls of 100 X 15-mm glass petri dish bottoms. For deployment, animals were transported to the spray sites and randomly placed in their respective cages. Either 3 or 4 cages containing from 7 to 10 mysids per cage (depending on day of deployment) were deployed. When placed in cages, mysids were 9-to 15-days old, and those deployed at a given time were the same age. Although sheepshead minnows were caged continuously during the study, they were deployed on only two occasions. Three cages containing 10 fish per cage were used for each deployment. Caged fish were from a population hatched over a 5-day period and were approximately 1-month old when used in the field study. Caged mysids and sheepshead minnows were not fed. Survival was monitored at least daily during the first four days after deployment.

Field site laboratory tests with pink shrimp - We tested each batch of shrimp purchased for field exposures to determine batch-to-batch vari-

ability in sensitivity to fenthion. Static, 48-h toxicity tests were conducted with reference site water in 19-ℓ (5-gallon) plastic buckets containing a 2-cm layer of clean beach sand and 16.5 ℓ of unfiltered seawater. A control, a carrier control (1.0 mℓ acetone added), and five fenthion concentrations (1.0, 0.75, 0.50, 0.25, and 0.15 µg/ℓ) were tested. Each treatment was replicated, with 5 shrimp per replicate. Gentle aeration was maintained in all buckets. Test salinity ranged from 28°/oo to 32°/oo among tests. Each test was conducted at 24° C.

RESULTS AND DISCUSSION

Toxicity Test Results

Laboratory pulse-exposure tests with mysids - Maximum concentrations of fenthion ≥ 5 µg/ℓ consistently killed 100% of exposed mysids; partial or complete kills occurred in mysid populations exposed to maximum concentrations from 1.1 to 3.0 µg/ℓ ; and for all exposures where fenthion peaked at < 1.0 µg/ℓ, no significant (≤ 10%) mortality occurred (Table 1). There was little difference in mysid mortality in pulse-exposure tests diluted at 15 or 30 ℓ/h. These data established a clearly defined, maximum pulse-exposure regime for which there were no acutely lethal effects. By using similar exposure regimes, tests with pink shrimp established a peak concentration of 0.84 µg/ℓ as the maximum no-effect fenthion concentration; maximum concentrations ≥ 4.0 µgℓ killed all exposed pink shrimp (1).

Field site laboratory tests with pink shrimp - The same bait-shrimp fishery that supplied pink shrimp for tests reported by Borthwick et al. (1) was used as the source for our field study animals. This eliminated adaptation by local animals as a source of variability in laboratory-field comparisons. Pink shrimp from all batches purchased during the field study were similar in their sensitivity to fenthion (Table 2). The

LC50's from static acute toxicity tests conducted during the field study ranged from 0.35 to 0.46 µg/ℓ in 24-h exposures and from 0.29 to 0.41 µg/ℓ in 48-h exposures (Table 2). These ranges bracket the 24-h and 48-h LC50's of 0.50 and 0.25 µg/ℓ, respectively, reported by Borthwick et al. (1) for static tests under controlled, laboratory conditions. Because mysids and sheepshead minnows used in field tests were from our own laboratory stocks, their sensitivity was assumed to be the same as those tested to derive LC50's reported by Borthwick et al. (1).

Field Test Results

A detailed description of test conditions, concentrations of fenthion in water and animal mortality following the two aerial applications for mosquito control will be reported elsewhere. Only a brief summary of data pertinent to laboratory-field comparisons is provided below.

Test conditions - Temperature, salinity, and dissolved oxygen (DO) at Site 1 reflected greater tidal exchange with Gulf waters than at Site 2. Temperatures ranged from 30°C to 33°C at Site 1 and from 27°C to 30°C at Site 2 during the 9-day study. Salinity ranged from 32°/oo to 35°/oo at Site 1 and 30°/oo to 33°/oo at Site 2. The DO at Site 1 ranged from 6.0 to 8.0 mg/ℓ, but DO at Site 2 ranged from 3.8 mg/ℓ to 4.5 mg/ℓ for early morning samples (6-8 AM) during the study period, or 56% to 71% of saturation. During other times of the day, DO ranged from 5.0 to 8.0 mg/ℓ, approximating or exceeding saturation. Based on the low mortality observed when fenthion was not present, neither the extremes nor rate of change of temperature, salinity, or DO is believed to have placed excessive stress on caged test animals.

Exposure concentrations - Both aerial thermal fog applications of fenthion produced a surface slick of diesel fuel which persisted for up

to 2 h. Initial concentrations of diesel fuel in water consistent with the maximum concentration of fenthion recorded would have been approximately 50 µℓ/ℓ, based on a 1:20 fenthion:diesel ratio. This concentration of diesel is more than a factor of 20 less than the 96-h LC50's for mysids and larval grass shrimp. Because of the relatively low concentrations and brief exposures, diesel fuel is not considered a confounding factor in our labortory-field comparisons of test animals exposed to fenthion.

The first fenthion application at Site 1 produced a surface concentration of 1.5 µg/ℓ and a bottom concentration of 0.36 µg/ℓ in samples taken 0.5 h after the airplanes had passed overhead. These concentrations diminished to 0.12 µg/ℓ surface and 0.070 µg/ℓ bottom by 2 h post-spray and to less than 0.010 µg/ℓ surface and 0.012 µg/ℓ bottom by 12 h. No fenthion was detected at Site 1 24 h after Spray 1. Spray 2 produced a peak fenthion concentration of 0.29 µg/ℓ, measured at the surface 6 h post-spray. Other water samples taken between 0.5 h and 24 h post-spray ranged from no fenthion detected to 0.15 µg/ℓ. For both sprays, initial fenthion concentrations in bottom water samples were consistently less than in surface water samples.

At Site 2, fenthion concentrations in the surface water following Spray 1 peaked at 2.6 µg/ℓ after 0.5 h, fluctuated between 0.39 and 1.4 µg/ℓ until 24 h post-spray, and then slowly diminished to 0.053 µg/ℓ by 60 h post-spray. Bottom water samples showed a slow increase in fenthion during the 60 h following Spray 1. Concentrations ranged from 0.028 to 0.13 µg/ℓ during this interval. Spray 2 produced initial peak concentrations of 0.51 and 0.36 µg/ℓ, surface and bottom. Surface concentrations then ranged from 0.23 to 0.14 µg fenthion/ℓ and bottom concentrations were from 0.29 to 0.15 µg/ℓ until 24 h post-spray. Over the subsequent 72 h, fenthion

concentrations for both surface and bottom water samples ranged from 0.087 to 0.036 µg/ℓ.

Animal mortality - No animal mortality attributable to fenthion poisoning occurred among animals deployed at Site 1. Survival of caged pink shrimp, mysids, and sheepshead minnows for 72 h post deployment was $\geq$ 90% for all groups. At Site 2, exposure to fenthion killed all surface-deployed pink shrimp within 24 h following Spray 1 and within 48 h following Spray 2. Mysid mortality was $\geq$ 88% 48 h after Spray 1 and $\geq$ 40% 48 h after Spray 2 for animals deployed the morning of a spray or 24 h prior. Mortality from 20% to 80% was observed for batches of pink shrimp and mysids deployed one or two days after the fenthion sprays. Mortality was $\leq$ 10% for pink shrimp and mysids deployed for 24 h before Spray 1 or 48 h after Spray 2; i.e. for intervals when fenthion was undetectable or detectable at low concentrations. All sheepshead minnows deployed at Site 2 survived the fenthion exposures.

Laboratory - Field Comparison

Pulse exposures - For laboratory tests to be predictive of field results, animal exposure regimes (concentration vs. time profiles) must be similar. The maximum concentration of fenthion measured at Site 1 following Spray 1 (1.5 µg/ℓ) and Spray 2 (0.29 µg/ℓ) exceeded or approached the 24-h LC50's for pink shrimp and mysids (0.40 and 0.42 µg/ℓ, respectively) (1). However, the field concentrations rapidly decreased to less than 0.020 µg fenthion/ℓ by 12 h post-spray and caused no deaths among caged animals. Laboratory-derived, pulse exposure data for pink shrimp (1) and mysids (this paper) and field exposure data from the two sprays at Site 1 are in excellent agreement (Fig. 1). The 100% mortality observed for pink shrimp and 44% mortality for mysids deployed at Site 2 during Spray

1 confirmed laboratory test predictions of acutely lethal pulse exposures to fenthion (Fig. 1). Similar agreement between laboratory and field pulse exposures for pink shrimp exposed to fenthion following mosquito control applications from truck-mounted equipment has been reported (1).

Extended exposures - Because flushing and subsequent dilution were limited at Site 2, concentrations of fenthion changed more slowly with time relative to changes at Site 1. Site 2 exposure regimes were more comparable to routine laboratory toxicity tests. Exposure-mortality observations from daily caged animal deployments at this site were divided into 24-, 48-, and 72-h data sets for comparison with 24-, 48-, and 72-h LC50's generated from laboratory tests. Pink shrimp deployments yielded 12 24-h data sets, 10 48-h data sets, and 8 72-h data sets for comparison with laboratory test results. For mysids, the numbers of data sets for comparison were 7, 6, and 3 for 24-, 48- and 72-h field exposures, respectively. The number of data sets differed between species because pink shrimp were deployed on both the surface and bottom, while mysids were caged only at the surface. There were differences among time periods because (1) 100% mortality occurred in a batch within 24 h so there was no 48- or 72-h exposure, or (2) animals were deployed for < 72 h during the later days of the field study. From the various exposure-response data sets available, those plotted in Figure 2 were selected to present responses that range from none observed to 100% mortality.

24-h exposures - The only 24-h exposure regime that caused 100% mortality of pink shrimp was that in surface water following Spray 1. All shrimp in the group deployed the day before the spray and all those

deployed the morning of the spray died (Fig. 2). For the group of mysids deployed the morning of Spray 1, 44% mortality occurred during the 24 h post-spray (Fig. 2), while the pre-spray group experienced 78% mortality. This exposure curve lies substantially above the 24-h LC50 for either species during the first 10 h post-spray and at the LC50 concentration for the next 14 h.

The 24-h exposure regime with the highest concentrations that caused $\leq$ 20% mortality among caged pink shrimp also is shown in Figure 2. Below this curve, there were six other exposures at Site 2 (not illustrated) that resulted in mortality $\leq$ 10% for caged pink shrimp. All of these no-effect or limited mortality exposure regimes peaked below the LC50 and diminished. Another 24-h exposure that caused significant mortality in pink shrimp followed Spray 2 when fenthion concentrations initially exceeded the LC50 (0.40 µg/ℓ) but decreased to approximately 0.20 µg/ℓ between 1 and 24 h post-spray. Although the exposure curve was substantially below the LC50 and the pulse exposure was below the no-effect profile (Fig. 1), 83% of the caged pink shrimp died.

Responses of mysids deployed after Spray 1 and exposed to fenthion for 24 h yielded mortality $\leq$ 17%, with three exposures resulting in $\leq$ 6% mortality. Mortality of caged mysids or lack of response over 24-h exposures agreed with results that would have been predicted from laboratory toxicity tests.

48-h exposures - Following Spray 2, all pink shrimp caged at the surface died within 48 h as the fenthion concentrations pulsed above, and then remained near the 48-h LC50 (Fig. 2). As the result of an exposure regime that started at nearly double the LC50, fell below it by 12 h, and continued to decline over the succeeding 36 h, 53% mortality occurred.

From an exposure regime (not illustrated) that persisted at the 48-h LC50 for 24 h and then declined slowly to 0.020 µg/ℓ during the next 24 h, 60% mortality occurred among caged pink shrimp. There were 5 exposure regimes that remained below the 48-h LC50 and produced $\leq$ 12% mortality.

Exposure to fenthion following Spray 1 produced 88% mortality in 48 h among mysids deployed the morning of the spray (Fig. 2) and 95% mortality among the pre-spray group (not illustrated). This exposure regime exceeded or equaled the 48-h LC50 for the initial 24 h of exposure, then diminished over the next 24 h. Following Spray 2, 40% mortality occurred among caged mysids when the initial pulse exceeded the LC50, decreased to approximately 0.20 ug/ℓ, then slowly diminished over the next 47 h. Mortality in another group of mysids was 16% from an exposure that started above the 48-h LC50, fell below it over 12 h, and rapidly diminished over the remaining 36 h (not illustrated). From two exposure regimes that remained substantially below the LC50, 7% mortality was observed in one group and no mortality in another group of caged mysids.

72-h exposures - Three 72-h exposure regimes that included fenthion pulses following Spray 2 resulted in mortality of 85% (Fig. 2) or greater (not illustrated) among caged pink shrimp. An exposure similar to the "A" curve illustrated and another that started and remained above the 72-h LC50 for 24 h, fell to concentrations at or below it for 24 h, then pulsed above the LC50 following Spray 2 caused 100% mortality in caged pink shrimp. Mortality was 59% among pink shrimp that experienced a 24-h exposure near the 72-h LC50, 24 h of decreasing fenthion concentrations, and the 24-h pulse following Spray 2. For an exposure regime where fenthion concentrations slowly increased for 24 h, remained just below the 72-h LC50 for 24 h, then decreased over 24 h, pink shrimp mortality

was 10%. From two other exposure regimes substantially below the 72-h LC50 (not illustrated), mortality was $\leq$ 10%.

Mortality was 84% among mysids exposed to fenthion concentrations greater than the 72-h LC50 for approximately 20 h, less than the LC50 for 28 h, then the 24-h pulse following Spray 2 (Fig. 2). For mysids exposed to the 24-h pulse following Spray 2 and the subsequent 48 h when fenthion concentrations remained substantially below the 72-h LC50, 53% mortality occurred. Mortality for a third group of mysids exposed for 72 h at concentrations below the 72-h LC50 was 10%.

Validated laboratory-based predictions - For all time intervals, field exposures that greatly exceeded the laboratory-derived LC50's produced substantial mortality among caged pink shrimp or mysids. Mortality was < 10% for field exposures that were sustained well below the LC50's. For caged animals exposed to concentrations at the LC50's or for exposures that fluctuated above and below LC50's, mortality ranged from 20% to 90%. For the few cases when field mortality was greater than expected based on laboratory-derived LC50's, the field exposure included an initial pulse above the LC50's, followed by concentrations less than the LC50 but still measurable.

Exceptions to the validated predictions - Only one batch of caged animals demonstrated field mortality substantially higher than expected from laboratory test results. Pink shrimp deployed at Site 2 the day after Spray 2 died in greater numbers than expected based solely on their exposure to fenthion (Fig. 3). All animals from the same batch deployed at Site 1, where fenthion concentrations were less than 0.05 µg/ℓ, and at the reference site survived for 72 h. Survival of mysids deployed at Site 2 during the same time interval equalled 90%. The unexpectedly

high pink shrimp mortality at Site 2 may have been caused by stress endured by the animals while being held overnight prior to field deployment. During the night before deployment, water flow to the holding tanks stopped and approximately 40% of the shrimp died. Under laboratory test conditions, the animals would not have been used and the test rescheduled. During an onging field study, however, the animals were used because no other replacements were available. At the reference site and Site 1, where physical and chemical conditions were ideal for survival, no mortality occurred. The higher-than-expected mortality at Site 2 suggests that stressed animals may be more sensitive to fenthion poisoning. This could be an important factor in field studies because sites of limited dilution and flushing are where pesticide concentrations can be maximal and water quality can be less than optimal.

CONCLUSIONS

Observations on survival and mortality of caged pink shrimp, mysids, and sheepshead minnows exposed to fenthion following mosquito control applications agreed with exposure-response relationships developed under laboratory conditions when field and laboratory exposure regimes were similar. We believe that interpretation of results was not confounded by the use of diesel fuel carrier for pesticide application. Routine acute toxicity test results overestimated the toxicity of fenthion to caged pink shrimp and mysids in a habitat with rapidly diminishing concentrations of fenthion. However, laboratory tests that employed pulse-exposure regimes similar to those observed in the field did provide reliable estimates of effect or no-effect fenthion concentration from field exposures to pink shrimp and mysids. Exposure to fenthion caused no mortality in caged sheepshead minnows and none was expected since fenthion concentrations in the field were three orders of magnitude less than laboratory-derived LC50's.

The application of results of this field validation study are limited to acute responses of caged animals. We made no attempt to quantify effects on non-caged animals or populations that resided within the canal system or along the sound, but qualitative observations on animal communities along the seawalls and in the canals indicated a diversity of animal life before and after fenthion applications. Research is needed to correlate risk assessment projections based on single species testing with effects on resident populations and communities. Validation of acute response predictions by using caged animals in the field is merely the first step towards validating risk assessment procedures.

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Table 1. Mortality of mysids (*Mysidopsis bahia*) following exposure to pulsed concentrations of fenthion for approximately 12 h under controlled, laboratory conditions. After a single pulse exposure, mysids were maintained in fenthion-free seawater.

Maximum concentration during pulse exposure (ug/ℓ)	Rate of dilution (ℓ/h) after attaining maximum conc.	Mysid mortality at 96 h
11.0	15	100%
6.3	15	100%
5.0	15	100%
3.0	30	90%
2.9	15	85%
2.6	30	100%
2.1	15	65%
1.9	30	60%
1.6	30	85%
1.2	30	75%
1.1	30	25%
0.98	15	0%
0.86	15	10%
0.46	15	0%
0.41	15	0%

Table 2. Acute toxicity of fenthion to different batches of pink shrimp purchased for field tests. LC50's were computed by probit analyses from static, nominal exposure concentrations. Tests were conducted at a field site laboratory in Collier County, FL.

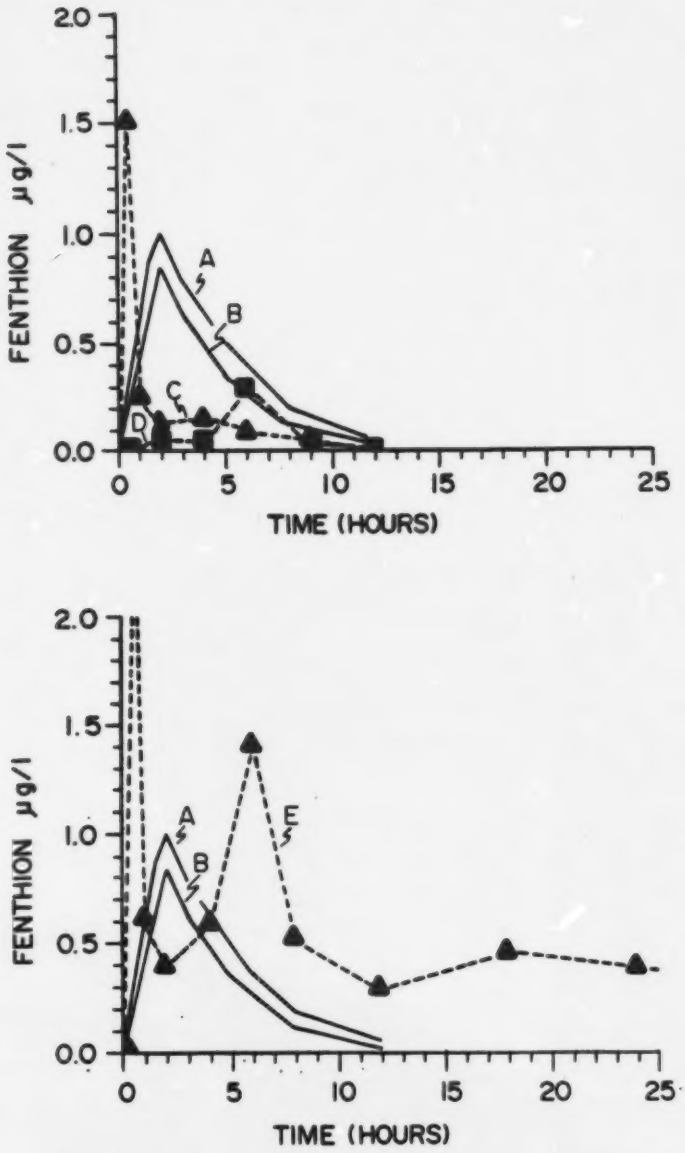
Test group by field deployment date	24-h LC50(µg/ℓ) (95% confidence interval)	48-h LC50 (µg/ℓ) (95% confidence interval)
6/19	0.35 (0.26-0.47)	0.35 (0.26-0.47)
6/20	0.39 (0.30-0.50)	0.35 (0.27-0.44)
6/23	0.46 (0.39-0.55)	0.41 (0.34-0.48)
6/25	0.39 (0.32-0.45)	0.29 (0.23-0.35)

Figure Legends

Figure 1. Laboratory-derived exposure-response regimes for 12-h pulses that resulted in no mortality in mysids (Mysidopsis bahia) (A) and pink shrimp (Penaeus duorarum) (B) compared to exposures of caged animals in the field when fenthion concentrations were changing rapidly. Top -- Following Sprays 1 (C) and 2 (D) at Site 1, fenthion concentrations were less than laboratory no-effect pulse-exposure concentrations. Survival of caged pink shrimp and mysids exceeded 93% following each Spray. Bottom -- Fenthion concentrations following Spray 1 (E) at Site 2 exceeded laboratory no-effect pulse-exposure concentrations and resulted in 100% mortality in caged pink shrimp and 44% mortality in caged mysids.

Figure 2. Mortality of caged pink shrimp (Penaeus duorarum) and mysids (Mysidopsis bahia) deployed for 24, 48, and 72 h on various dates at Site 2 and exposed to fenthion following Spray 1 or Spray 2. Mortality listed in upper right of each plot corresponds to the exposure regime measured for the interval of deployment. LC50's for each time period are plotted as a straight horizontal line.

Figure 3. Mortality of caged pink shrimp (Penaeus duorarum) exposed to fenthion for 48 h at Site 2. During the night before deployment, aeration to the holding system failed and 40% of the test animals died. Mortality from field exposures, listed in upper right corner, was greater than what would have been predicted from laboratory-derived LC50's.



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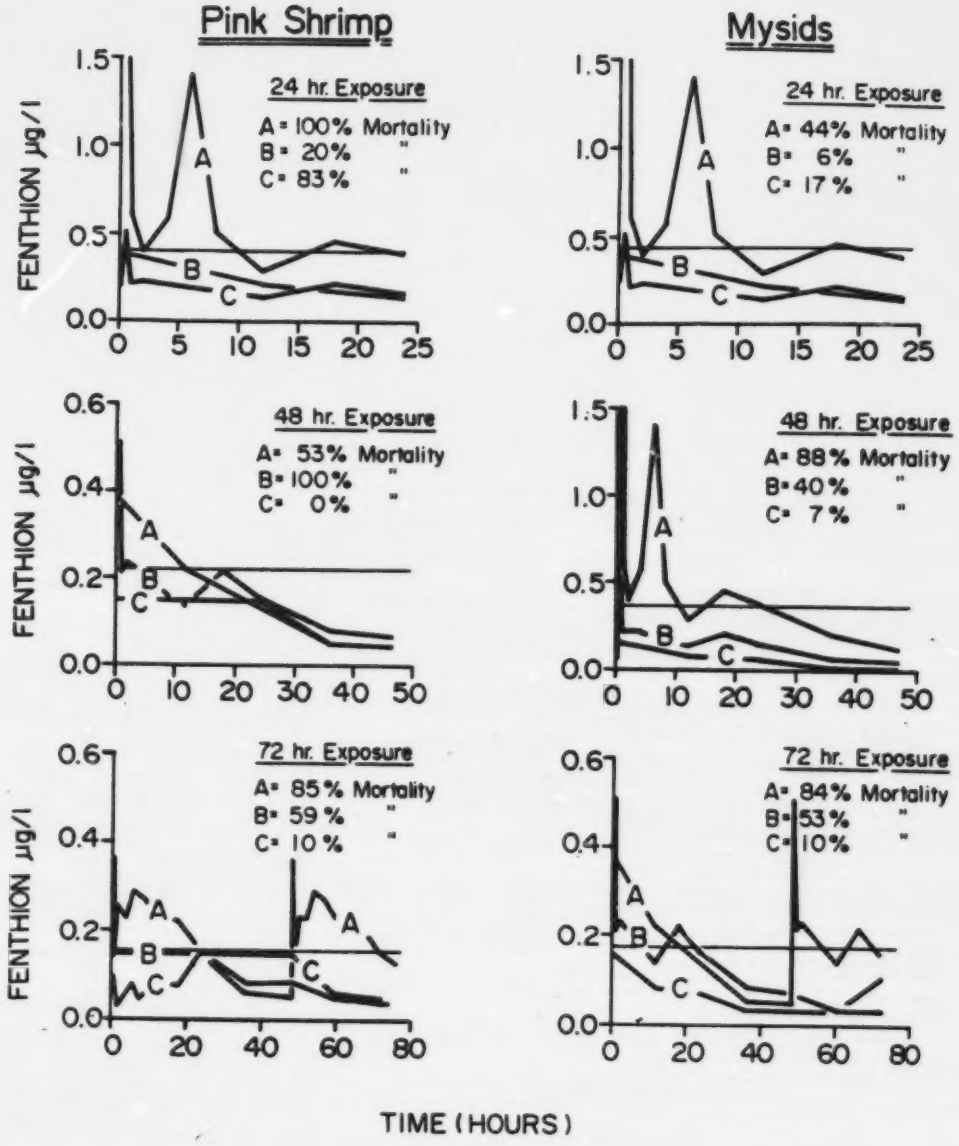

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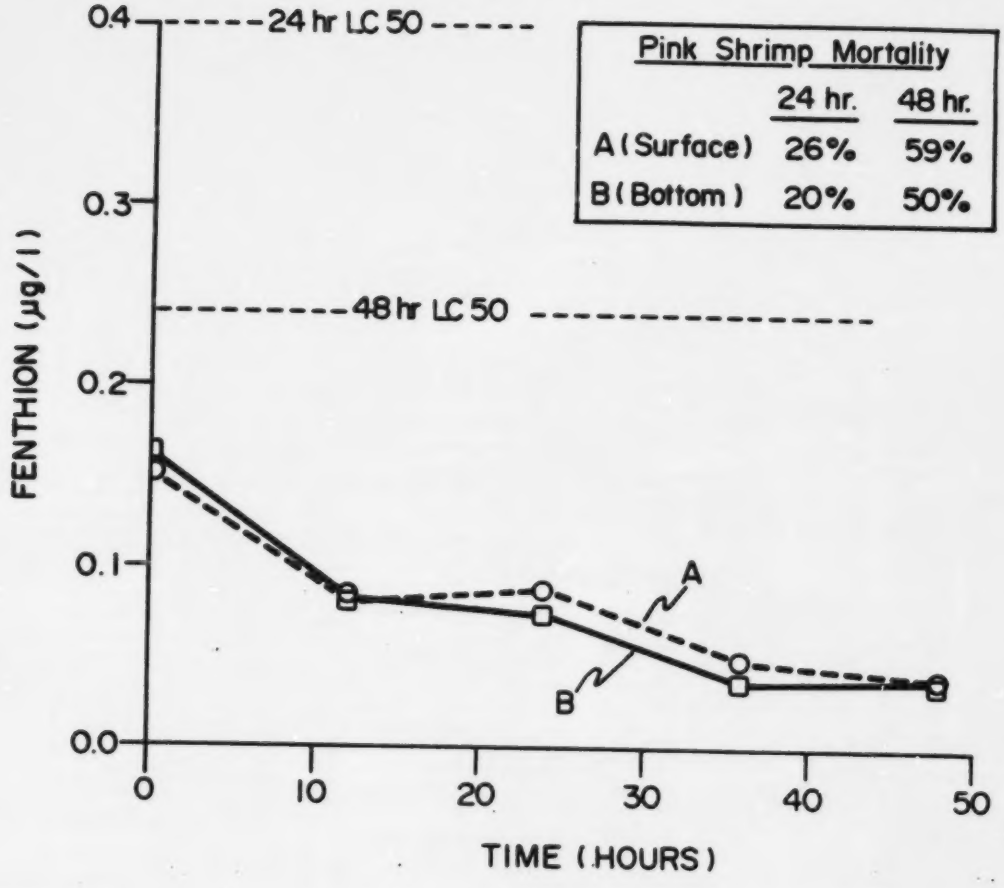

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